

***ANOPHELES (CELLIA) KOKHANI*, N. SP. (DIPTERA: CULICIDAE) FROM
KAPIT, SARAWAK, EAST MALAYSIA**

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Abstract.—*Anopheles kokhani* Vythilingam, Jeffery, and Harbach, n. sp., a member of the Neomyzomyia Series of subgenus *Cellia* Theobald (Diptera: Culicidae) is described from females collected in Sarawak, Malaysia, during studies to identify the mosquitoes responsible for transmitting simian malaria to humans. The species is distinguished from sympatric members of the Neomyzomyia Series, including *An. saungi* Colless, *An. stookesi* Colless, *An. tessellatus* Theobald, and *An. watsonii* (Leicester), with which it shares one or more key features. The wing and legs of the new species are illustrated.

Key Words: *Anopheles kokhani*, mosquito, new species, Neomyzomyia Series

The present paper describes a new species of *Anopheles* mosquito (Diptera: Culicidae) that was collected (along with 11 other species of the genus) during the course of studies to identify the vector(s) of *Plasmodium knowlesi*, a simian malarial protozoan recently found in humans (Singh et al. 2004). The study was conducted in Kapit, Sarawak, Malaysia, from June 2005 to April 2006. Bare leg catch (BLC) and monkey baited trap (MBT) methods were used to capture mosquitoes in forest, and BLC only inside and outside a longhouse (a dwelling used by local people) and in a farm at the forest fringe. The collections included 144 females of an unknown *Anopheles* species that could not be identified using the keys of Reid (1968) for the “Anopheline Mosquitoes of Malaya and Borneo.” The specimens also could not be

identified using keys for neighboring areas, including continental Southeast Asia (Rattananarithikul et al. 2006), the Philippines (Cagampang-Ramos and Darsie 1970) and the Australasian Region (Lee et al. 1987). Based on available character data, the specimens appear to be conspecific with the single female collected in the 3rd division of Sarawak that Cheong and Pillai (1965) recognized as a possible new species. Reid (1968) examined the single female, which was “reared from a larva found in a forest swamp,” and stated “Probably this is a new species but until specimens with larval and pupal skins are available it is not possible to be sure.” Although efforts to obtain progeny broods from blood-fed females and to find the immature stages of this species were unsuccessful, there is no doubt that the specimens

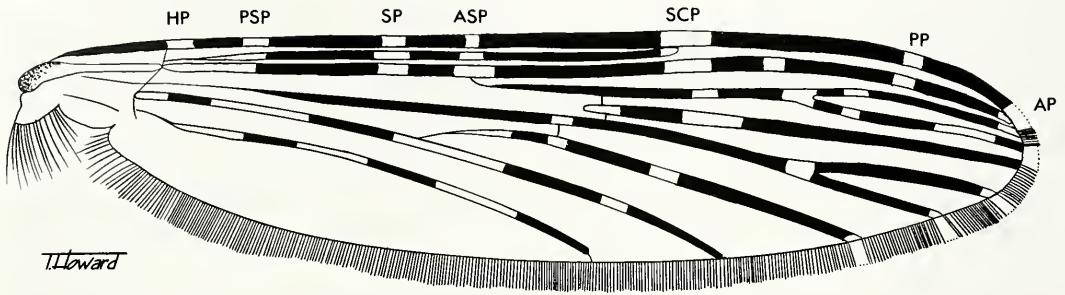


Fig. 1. Schematic outline drawing showing the pattern of pale and dark markings on the wing (dorsal surface) of *Anopheles (Cellia) kokhani* (female). Abbreviations for costal wing spots: AP, apical pale; ASP, accessory sector pale; HP, humeral pale; PP, preapical pale; SCP, subcostal pale; SP, sector pale. See the text, Reid (1968), and Harbach and Knight (1980) for the nomenclature applied to veins.

captured at Kapit represent a morphologically distinct species that is new to science. Consequently, this species is formally described and named *An. kokhani* n. sp. in this report. It is a pleasure to dedicate this species to Dr. Ng. Kok Han, Director of the Institute for Medical Research, Kuala Lumpur, in recognition of his tremendous support for the Institute's research activities.

MATERIALS AND METHODS

This study is based on the specimens mentioned above, 129 of which were subsequently dissected during vector incrimination studies. Specimens were examined under artificial and simulated natural light. Except for wing spot nomenclature (Wilkerson and Peyton 1990), the morphological terminology used in the species description follows Harbach and Knight (1980, 1982). Reid's (1968) system of numbering wing veins is used along with standard terms to aid workers in Malaysia. Diagnostic and differential characters were confirmed in all available specimens.

TAXONOMIC TREATMENT

Anopheles (Cellia) kokhani Vythilingam, Jeffery, and Harbach, n. sp.

(Figs. 1–2)

Diagnosis.—Females of *An. kokhani* are distinguished from other members of

the *Neomyzomyia* Series in the Oriental Region in having four pale bands on the maxillary palpus [distinction from *An. kolambuganensis* Baisas], anal vein of the wing with three dark marks [distinction from *An. tessellatus* Theobald; members of the *Leucosphyrus* Group], speckled legs [distinction from *An. aurostris* (Watson)], hindleg without broad pale band covering the tibio-tarsal joint [distinction from members of the *Leucosphyrus* Group], hindtarsi mainly dark-scaled with narrow apical pale bands [distinction from *An. kochi* Dönitz, *An. watsonii* (Leicester), *An. stookesi* Colless; *An. saungi* Colless], abdominal terga without scales [distinction from *An. kolambuganensis*], abdominal sterna without scale-tufts [distinction from *An. kochi*].

Female.—*Head*: Vertex with white erect scales behind frontal tuft, black erect scales posteriorly; frontal tuft with 9 or 10 long white sinuous setae. Clypeus bare. Antenna slightly longer than proboscis; pedicel without scales; flagellomere 1 with inconspicuous elongate gray to black scales on mesal surface, setae of flagellar whorl 2–3 times as long as flagellomeres. Proboscis 1.60–1.75 mm, about same length as forefemur; prementum entirely dark-scaled, scales semi-erect at base; labella pale. Maxillary palpus slightly shorter than proboscis, length 1.50–1.65 mm, with 4 narrow pale



Fig. 2. Legs (anterior aspects) of *Anopheles (Cellia) kokhani* (female) showing the pattern of pale markings: A, foreleg; B, midleg; C, hindleg.

bands, apical pale band about length of preapical dark band, other bands at apices of palpomeres 2, 3 and 4; palpomere 2 with semi-erect scales imparting a shaggy appearance to proximal portion of palpus. *Thorax*: Integument dark brown to black, scutum with mottled pattern of gray pruinose and darker areas, scutellum pruinose with median dark area contiguous with similar spot on prescutellar area of scutum; anterior promontory and antedorsocentral areas with white semierect scales that grade into yellowish to golden fusiform scales on anterior acrostichal area, some scattered pale scales on scutal fossa; bronze to black setae on acrostichal, dorsocentral, fossal, antealar, supraalar and prescutellar areas; sparse rather inconspicuous pale scales among antealar and supraalar setae. Scutellum with posterior row of long bronze to black setae interspersed with few fine golden setae, with cluster of fine golden setae medially. Mesopostnotum and postpronotum bare. Anteppronotum with or without few pale scales ventrally among few rather short golden-brown setae and few dark scales dorsally among long dark setae. Pleura dark with variable pruinose areas, with few golden-brown to black setae on proepisternum (1-3), prespiracular area (1-3), prealar knob (2-4), upper (2-4) and lower (3-5) mesokatepisternum and upper mesepimeron (3-5); lower mesepimeral setae absent. *Wing* (Fig. 1): Length about 3.2 mm; dark scaling black, pale scaling pale yellow, pattern of spots well defined, pale spots fairly narrow except on cubitus (veins 5 and 5.2 of Reid 1968) and anal vein (vein 6); costa with or without prehumeral pale spot, with humeral, presector, sector, accessory sector, subcostal, preapical and apical pale spots; remigium pale-scaled; vein R + R₁ (vein 1) like costa except presector pale spot extends to humeral crossvein, sector dark spot slightly shorter, sub-

costal pale spot slightly longer and preapical dark spot with pale interruption; $R_s + R_{2+3}$ (vein 2) mainly dark-scaled with indefinite pale spots at radio-medial crossvein (cv 2-3) and middle and apex of R_{2+3} , R_2 and R_3 (veins 2.1 and 2.2) with indefinite postbasal pale spots, R_2 with small apical pale spot and R_3 with relatively large subapical pale spot; R_{4+5} (vein 3) with small basal and slightly larger postbasal pale spots; $M + M_{2+3}$ (vein 4) dark-scaled with pale spot at fork of M_1 and M_2 (veins 4.1 and 4.2), M_1 and M_2 each narrowly pale at apex; mediocubital crossvein (base of vein 5.1) bare proximally, with dark scales distally; M_{3+4} (vein 5.1) with small pale spots at mediocubital crossvein, near mid-length and at apex; CuA (veins 5 and 5.2) with 4 dark spots: small well-defined spot near base, large spot before mediocubital crossvein and 2 large narrowly separated distal spots (distal spots sometimes fused to form a line of dark scales to wing fringe); 1A (vein 6) with 3 dark marks, apex without scales; apical pale fringe spot present between apices of veins R_3 and R_{4+5} and faint pale fringe spots usually visible at apices of M_1 , M_2 , M_{3+4} and sometimes CuA. *Halter*: Pedicel bare, pale; scabellum and base of capitellum with narrow white spatulate scales, dome of capitellum with smooth shiny covering of minute decumbent black scales that contrast sharply with adjacent white scaling. *Legs* (Fig. 2): Coxae without scales; trochanters with mixture of pale and dark (mainly or all dark) scales on ventral surfaces; femora, tibiae and first tarsomeres with speckles and blotches of pale yellow scaling, hindfemur and all tibiae with narrow knee spots; tarsomeres 1-4 of all legs with narrow apical pale bands, bands usually faint or obsolete ventrally on fore- and midlegs. *Abdomen*: Integument dark with long dark setae; scales absent except for narrow golden scales on posterior area of tergum VIII and dorsal

and lateral surfaces of cerci; sternum VIII with 2 or 3 narrow pale spatulate scales.

Male, pupa, larva, and egg.—Unknown.

Systematics.—*Anopheles kokhani* belongs to the *Neomyzomyia* Series of subgenus *Cellia* Theobald. Females key to couplet 17 in Reid's (1968) key to the anopheline mosquitoes of Malaya and Borneo. This couplet distinguishes *An. tessellatus* from *An. saungi*, *An. stookesi*, and *An. watsonii*, but *An. kokhani* cannot be identified as any one of these species based on key characters. *Anopheles kokhani* resembles *An. tessellatus* in having narrow apical pale bands on the hindtarsomeres, but differs in having the proboscis completely dark-scaled. These species also exhibit significant differences in characteristics of the maxillary palpus, proboscis, wings, and halteres, and do not appear to be closely related. *Anopheles kokhani* resembles the other three species in having a dark-scaled proboscis, but differs in having hindtarsomeres 3-5 mainly dark-scaled. These tarsomeres are pale-scaled in *An. stookesi* and *An. watsonii*, and hindtarsomere 3 is largely pale-scaled in *An. saungi*. *Anopheles kokhani* otherwise closely resembles these three species in overall habitus. It appears to share a closer relationship with *An. watsonii* than to *An. saungi* and *An. stookesi* based on shared features of the wings. Whereas vein R_{4+5} (vein 3 of Reid 1968) is nearly completely dark-scaled in *An. kokhani* and *An. watsonii*, it is largely pale-scaled in the other two species. Other characters, e.g., a pale fringe spot at the apex of vein CuA (veins 5 and 5.2 of Reid 1968), are too variable to distinguish the species or suggest possible evolutionary affinities.

Reid (1968) indicated that the Philippine *An. kolambuganensis* might be found in Borneo. Females of this species also run to couplet 17 in Reid's key, but are distinguished from *An. tessellatus*, *An.*

saungi, *An. stookesi*, and *An. watsonii*, as well as *An. kokhani*, by the presence of broad pale and dark scales that form "incipient" lateral tufts on the posterior corners of abdominal terga V–VII.

Bionomics.—*Anopheles kokhani*, *An. stookesi*, and *An. watsonii* all occur in Sarawak. Whereas *An. watsonii* was collected at the type locality of *An. kokhani*, *An. stookesi* is a highland species, having been collected above 610 m (Reid 1986, as 2,000 feet), and may not occur in sympatry with the other two species. *Anopheles saungi* is also unlikely to occur in sympatry with *An. kokhani* and *An. watsonii* as it has only been found above 1070 m in the Crocker Range of Sabah (Reid 1986, as 3,500 feet).

Females of *An. kokhani* and *An. watsonii* were collected at the same sites, albeit in different numbers, during the vector incrimination studies mentioned above. Collections in forest yielded 17 *An. kokhani* (15 in BLC and two in MBT collections) and 190 *An. watsonii* (121 BLC; 69 MBT). Collections made inside and outside a longhouse located about 5 km from the forest fringe on land cleared of original forest and now supporting fruit trees and secondary vegetation, included 46 *An. kokhani* (19 captured indoors and 27 outdoors) and only two *An. watsonii* (one indoors; one outdoors). Although the larval habitats of *An. kokhani* are unknown and may be more numerous outside forest than those occupied by *An. watsonii*, the collection data suggest that *An. kokhani* is the more endophilic species. BLC collections at a farm located closer to forest yielded 81 *An. kokhani* and one *An. watsonii*. Although both species are attracted to humans, the results show that *An. kokhani* is more peridomestic and anthropophilic than *An. watsonii*, which appears to be mainly a forest species. Most of the *An. kokhani* females (129) collected during the field studies noted

above were dissected for possible vector incrimination, but no sporozoites or oocysts of *Plasmodium* species, and no microfilariae, were found (unpublished observations).

According to Reid (1968), the presumed female of *An. kokhani* reported by Cheong and Pillai (1965) was reared from a larva found in a forest swamp. In contrast, larvae of *An. watsonii* are "usually found in fallen split, or cut-open bamboo, sometimes in collections of water on fallen tree trunks" (Reid 1968). This clearly indicates a demarcation in the habitat requirements of the two species.

Type series.—Fifteen females. *Holotype*, ♀ (no. 33), MALAYSIA: Sarawak, Kapit, 20 Apr 2006 (Vythilingam et al.). *Paratypes* (same locality and collectors as holotype), 14 ♀ (nos. 1, 10, 12, 16, 17, 24, 44, 55, 57a, 57b, 64, 68, 69, 90) collected between Jun 2005 and Apr 2006. The holotype female and two paratypes (nos. 12 and 24) are deposited in The Natural History Museum (NHM), London; two females (nos. 1 and 16) are to be deposited in National Museum of Natural History, Washington, D.C.; two females (nos. 10 and 57a) are to be deposited in Sarawak Biodiversity Council, Kuching, Sarawak, Malaysia; and the remaining 8 paratypes (55, 17, 68, 69, 90, 64, 44, 57b) are retained in the Institute for Medical Research, Kuala Lumpur, Malaysia.

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